Separation of amino acids by thin-layer chromatography

Mixtures of amino acids can be separated by using of silica gel chromatographic plate. The separated amino acids are visualized using solution of ninhydrin. Purple color develops upon reaction of amino acid with ninhydrin.

Aim of the work

Qualitative analysis of amino acids in given mixture.

Materials needed

1. Eluent: n-butanol, acetic acid(glacial) and distilled water in volume ratio 4:1:1.
2. Solution of ninhydrin. (Dissolve 0.3 g of ninhydrin in 100 ml n-butanol. Add 3 ml of glacial acetic acid.)
3. 0.02 M solutions of amino acids (leucine and serine). (Dissolve: 0.026 g leucine and 0.021 g serine in distilled water and bring the volume to 10 ml.)
4. Silica gel plates with dimensions 80 x 40 mm
5. Elution reservoir
6. Glass capillaries for spotting the samples.
7. Graduated test-tube.
8. Solution of ninhydrin in spraying bottle.
9. Graphite pencil,ruler, scissors and rubber gloves.

Procedure

1. Mark the starting line to the plate 1 cm from the edge of the plate - with graphite pencil.
2. The spots of individual amino acids, also their mixture solutions are applied to the chromatographic plates.

Do not wet the silica beyond a diameter of 2-3 mm. After the liquid has evaporated (only a few seconds), add a second drop to the same spot. After application of samples let the spots dry. Pour 5 ml of eluent into the elution reservoir. Cover the chamber with lids and let the chamber atmosphere saturate with eluent vapours for 10 min. To start the analysis, insert the silica gel plate in reservoir and cover it with lid. Elution is stopped when the solvent front has traveled up the plate until 7-10 mm from the top of the plate.

3. Remove the plate from elution reservoir and quickly mark solvent front with a pencil.
4. Dry the plate. Be careful to move the heat gun around and not heat one point continuously. Do this procedure in the hood.
5. Spray the plate with the ninhydrin solution. Plate should lie at 45° angle while spraying. Dry the plate again.

6. Dry the plate again with the heat gun. Do not over heat the plate. Long heating may cause browning of the plate over the entire surface. Circle each colored spot with a pencil.
7. Measure the distance from the origin to the center of each
colored spot and calculate the Rf values for all spots.

R_f = distance traveled by amino acid/distance traveled by solvent